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PATENTS ACT 1990

PROVISIONAL SPECIFICATION

FOR THE INVENTION ENTITLED:-

"Methods for diagnosing pre-cancerous and cancerous conditions."

The invention is described in the following statement:-

TECHNICAL FIELD

The present invention relates to methods of identifying pre-neoplastic and/or neoplastic states in mammals and in particular to a method for identifying pre-neoplastic and neoplastic cells in tissues and body fluids, based on differential expression of purinergic receptors in these cells.

BACKGROUND

When diagnosing cancer, cellular features in biopsy samples are taken into account such as, the degree of variability of cancer cell size and shape, the proportion of actively dividing cells and invasion into neighbouring structures. Commonly used histological stains are haematoxylin (primary stain) and eosin (counterstain) which differentially label subcellular elements. Other diagnostic methods employ antibodies to particular diagnostic molecules within (via intracellular epitopes) or on the surface of cells or tissues (via extracellular epitopes) which can be made visible for microscopic analysis eg, carcino-embryonic antigen (CEA). Some specific examples are discussed below.

Prostate Cancer

The incidence of prostate cancer in the Western world is increasing at an alarming rate, having more than doubled in the past five years. It has the highest incidence of any neoplasm. is second only to lung cancer as the most common cause of cancer death in men worldwide, and is the leading cause of death in Australia [1]. Benign prostatic hyperplasia (BPH) is common in men over 50 and is a possible precursor of prostatic intraepithelial neoplasia (PIN), itself a precursor to prostate cancer. Postmortem studies indicate that 70% of men have malignant cells in their prostate by the time they reach 80 [2]. This disease is characterised by a striking

racial variation and is most prevalent in African-Americans, intermediate in Caucasians, slightly lower in Latinos, and least prevalent in Asians. In the latter group, it is nevertheless the most rapidly increasing form of neoplasm. Until recently, it was not clear if these differences were due to racial genetic variation or diet.

5   Studies have now shown that diet is a primary influencing factor [3].

Current diagnosis and treatment of prostate cancer

Despite the gravity of this condition, diagnostic methods are few and imprecise. Current methods for assessing prognosis such as digital rectal examination (DRE), ultrasound, prostatic acid phosphatase levels, androgen ablation, prostate

10   specific antigen (PSA) density, PSA velocity, PSA age-specific reference ranges and Gleason histopathological grading, can fail to provide reliable predictive information regarding the clinical outcome of prostate cancer [4]. For instance, studies have shown that DRE results in a 36.9% false negative rate [5]. PSA is a 33-kDa serine protease that is associated with a number of tissues besides prostate [6], is up-

15   regulated by androgens, glucocorticoids and progestins and is thought to be involved in the regulation of growth factors. Unfortunately, serum PSA levels have an incidence of 23% false negative and 36.7% false positive diagnoses [6]. It has even been suggested that more than half of new screen-detected cases are in fact false positives [7]. Attempts to improve screening methods by the introduction of

20   additional tests such as PSA density, velocity, and age-specific reference ranges has been equivocal. One study has shown that applying an age-specific PSA reference range that increases the upper limit of normal PSA to 4.5 ng/mL results in the failure to detect a substantial number of clinically significant cancers [8]. Given this

uncertainty, prostate biopsy is often performed to confirm malignancy but this test

also has a highly unsatisfactory 23% incidence of false-negative diagnosis [9].

Treatment selection is largely dependent on clinical staging based on

microscopic analysis of tissue sections [10]. This technique depends on judgment and

5   considerable experience in relating histological appearance to clinical outcome.

Unfortunately, prostate cancer tissue is notoriously heterogeneous and a vital

diagnostic feature may easily be missed in the section being examined. To further

complicate the situation, there have been no randomised and controlled trials to

examine the outcomes of surgery and radiotherapy [2]. Treatment choices include

10   radical prostatectomy, radiation therapy, androgen deprivation and "watchful

waiting". A definitive answer to the question of "watchful waiting" versus radical

intervention awaits the conclusion of the prostate cancer intervention-versus-

observation trial [11]. The consequences to the patient of these decisions are serious.

Radical prostatectomy for instance, often results in incontinence, impotence, bladder

15   neck stricture and depression [12]. Clearly, improved markers that reliably

differentiate between benign prostatic hyperplasia (BPH), prostatic intraepithelial

neoplasia (PIN), atypical adenomatous hyperplasia (AAH) and prostatic cancer are

urgently needed.

The role of P2X receptors in cancer

20   Neurotransmitters such as noradrenalin and acetylcholine act not only in the

synapse and neuromuscular junction but also on transmitter-specific cell receptors in

a wide variety of tissues and organs. These receptors are pore-like transmembrane

channels that introduce ions into the cell. Adenosine triphosphate (ATP), best known

as the molecular currency of intracellular energy stores, was first proposed as a

peripheral neurotransmitter based on its ability to contract smooth muscle [13]. ATP

acts in the same manner as other neurotransmitters and can activate both the

(relatively slow) G protein-coupled tissue receptors (P2Y), the more recently

characterised (fast) ligand-gated purinergic (P2X$_{1-7}$) ion channels and can also act as a

5   co-transmitter. Despite its relatively recent discovery, it is likely that the purinergic

transmitter system developed very early in evolution [14].

There are currently 7 genetically distinct P2X receptor subtypes. They are as

widely distributed as receptors of the cholinergic and adrenergic systems and are

found in most mammalian cells [14]. These receptors constitute a new class of fast-

10   response, membrane-bound, ligand-gated, calcium-permeable, cation-selective

channels that are activated by extracellular ATP from nerve terminals or a local tissue

source [15-18]. They are predominantly permeable to calcium ions but also admit

other cations, such as potassium and sodium, thereby mediating depolarisation [19].

For instance, in lung epithelia, P2X channels stimulate Cl$^-$ channel up-regulation, K$^+$

15   secretion and inhibit Na$^+$ absorption (21). ATP can stimulate both DNA synthesis

and cell proliferation via the up-regulation of the P2X receptors [14]. This function is

linked to stimulation of phospholipase C and ionic calcium release from inositol-

phosphate-sensitive intracellular stores, as well as other signal transduction pathways.

These actions are potentiated by the synergistic action of ATP with polypeptide

20   growth factors [20]. The influx of calcium through the P2X receptors also triggers

the secretion of other neurotransmitters, serves as a signal for the activation of

calcium-dependent potassium channels, inactivates other calcium channel types,

regulates endocytotic retrieval of synaptic vesicle membranes, enhances the synthesis

of neurotransmitters, regulates pools of synaptic vesicles available for secretion and triggers several forms of synaptic plasticity. The variety of responses to a single stimulation of P2X receptors suggests there are many calcium-activated pathways [21].

5   Extracellular ATP, acting via the purinergic receptors, also has a direct anticancer effect on human breast cancer cells, prostate carcinoma cells, human adenocarcinoma cells and fibroblast cell lines. Cytotoxic T lymphocytes and natural killer (NK) cells release ATP when they attack tumour cells [22]. Only transformed cell growth is inhibited, by inducing S phase block, apoptosis, increased permeability

10   to nucleotides, sugar phosphates, ions and synergy with other anticancer agents. None of these effects are noted on untransformed cells [14].

Curiously, tumour cells are known to contain exceptionally high levels of ATP [23]. Adenosine and ATP both increase intratumour blood flow by stimulating nitric oxide synthesis from the endothelium, thus inducing potent vasodilation [24].

15   In this case ATP acts through P2Y receptors (26). Nitric oxide release is also linked to P2X receptor function. For instance, 90% of the nitric oxide synthase activity found in non-pregnant sheep myometrium is calcium ion-channel dependent [25].

Epithelial adhesive proteins also play a major role in the spread of cancer [26]. In wound healing, cell injury signals propagate via extracellular P2X receptors and

20   intercellular gap junctions. stimulating calcium ion-induced wave propagation [27]. Intracellular calcium ions admitted by the P2X channels trigger the transport of membrane-bound organelles along microtubules, remodelling of the ECM and up-regulation of the adhesion molecule E-cadherin [28]. The myoepithelial cells found in prostatic epithelial acinar exert important paracrine effects on carcinoma cells both

in situ and *in vitro*. Cancer cells are also affected by high expression of ECM molecules, proteinase inhibitors and angiogenic inhibitor [29]. During metastatic invasion, extracellular calcium influx activates membrane-associated metalloproteinases that facilitate tissue penetration by invasive cells. Urokinase

5   plasminogen activator has also been strongly implicated in the progression of several malignancies including breast and prostate cancer [30].

Current techniques for staging and diagnosing cancer need to be improved in order to provide more reliable results using relatively simple technology. It would also be advantageous to have a diagnostic method amenable to automation.

10   It is an object of the present invention to provide a method of identifying pre-neoplastic and/or neoplastic cells which will overcome or substantially ameliorate at least some of the deficiencies of the prior art or will provide a useful alternative.

SUMMARY OF THE INVENTION

The purinergic nervous system operates in parallel with the better known but

15   slower acting adrenergic and cholinergic nervous systems. Like them, it operates in the brain, synapse, neuromuscular junction, peripheral nervous system and smooth muscle. The transmitter substance activating these fast-acting ligand-gated cation receptor channels is ATP, which acts by triggering purinergic receptors in tissues, resulting in a variety of metabolic responses including an influx of ions into the cell.

20   A unique suite of highly specific antibodies able to differentiate between the extracellular domains of each of the P2X purinergic receptor subtypes has been developed. These receptors are readily visualised using immunocytochemical methods and present in a variety of expression patterns such as cell surface, tubular and punctate labelling. It has surprisingly been shown that the expression of P2X

receptors is characteristic for pre-cancer and cancer stages and also for tissue from young vs old mammals. These changes are accompanied by marked differences in growth, extracellular matrix, metabolic and innervation factors as well as increases in subepithelial ionic calcium and microtubules. The invention therefore provides a new

5   tool with which to diagnose pre-cancerous conditions, (such as hyperplasia), stage cancer and to investigate the basic physiology and aetiology of carcinogenesis.

According to a first aspect, the invention provides a method of staging and/or diagnosing pre-neoplastic and/or neoplastic states in a mammal, including detection of the P2X purinergic receptor expression profile of cells and/or tissue from said

10   mammal and comparison of the profile with a predetermined expression profile of normal cells and/or tissue.

According to a second aspect, the invention provides a method of determining the aetiology of carcinogenesis in a mammal, including detection of the P2X purinergic receptor expression profile of cells and/or tissue from the mammal and

15   comparison of the profile with a predetermined expression profile of normal cells and/or tissue.

According to a third aspect, the invention provides use of a P2X purinergic receptor antibody reagent to stage and/or diagnose a pre-neoplastic and/or neoplastic state in a mammalian subject, including detection of the P2X purinergic receptor

20   expression profile of cells and/or tissue from the subject and comparison of the profile with a predetermined expression profile of normal cells and/or tissue.

According to a fourth aspect, the invention provides use of a P2X purinergic receptor antibody reagent to determine the aetiology of carcinogenesis in a mammalian subject, including detection of the P2X purinergic receptor expression

profile of cells and/or tissue from the subject and comparison of the profile with a predetermined expression profile of normal cells and/or tissue.

According to a fifth aspect, the invention provides an isolated mammalian cell or tissue sample complexed with a P2X purinergic receptor-specific antibody reagent.

5   According to a sixth aspect, the invention provides a kit for diagnosing a pre-neoplastic and/or neoplastic state in a mammal including means for detecting P2X purinergic receptor expression level in a sample containing cells and/or tissue from the mammal and means for comparing the expression level with a predetermined expression level.

10   According to a seventh aspect, the invention provides an antibody reagent specific for a P2X purinergic receptor, wherein the reagent is capable of differentiating between pre-neoplastic or neoplastic cells and/or tissue and normal cells and/or tissue.

According to an eighth aspect, the invention provides an antibody reagent

15   specific for a P2X purinergic receptor when used to differentiate between pre-neoplastic or neoplastic cells and/or tissue and normal cells and/or tissue.

Preferably the mammal is a human although it will be clear to the skilled addressee that the method may be applied to any mammal. Preferably the cells are prostate tissue cells or breast tissue cells. The cells may be obtained by biopsy but

20   may also be obtained from digital rectal examination exudate in the case of prostate tissue cells or from semen.

Preferably the antibody reagent includes a polyclonal antiserum. Preferably the P2X antibody reagent is specific for $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$ or $P2X_7$ receptors, most preferably $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$ or $P2X_6$ receptors and even

more preferably $P2X_1$, $P2X_3$ or $P2X_4$ receptors. It will be clear to those skilled in the art that the antibody reagent may be a suite of antibodies that may be polyclonal or monoclonal. It will also be clear to those skilled in the art that the suite of P2X receptor antibodies may include any combination of the P2X receptor subtypes, and

5   in particular the combination of $P2X_1$, $P2X_3$ and $P2X_4$.

Preferably detection of P2X receptor expression profile is by immunohistochemical means. It will be clear to the skilled addressee that the P2X receptors may be detected by other means including ELISA, RIA or similar immunological techniques, depending on the source of the cell or tissue sample and

10   the reagents available. Preferably, the P2X receptors are detected by a colorimetric assay. It will also be clear to those skilled in the art that Western blotting techniques and detection of P2X purinergic receptor mRNA may be useful in determining the P2X receptor expression profile.

In the context of the present invention, the term "pre-neoplastic cells"

15   includes cells that are hyperplastic. The term "hyperplastic" will be understood to include within its meaning "hypertrophic".

In the context of the present invention the term "suite of antibodies" includes polyclonal antibodies which contain several different antibodies specific for the same or different antigens and which are able to specifically differentiate between each of

20   the P2X receptor subtypes. When the antibodies are monoclonal, the term "suite of antibodies" also includes a panel of antibodies able to specifically differentiate between each of the P2X receptor subtypes.

BRIEF DESCRIPTION OF FIGURES

Figure 1 shows an example of the level of $P2X_1$ labelling in a biopsy sample taken from a normal human prostate (left) and from a patient with advanced prostate cancer (right).

5   Figure 2 shows a comparison of prostate epithelium (E) from a young (12 week) rat (left), and tissue from an aged rat (18 months; right). The aged tissue shows marked hyperplasia.

Figure 3 shows an example of $P2X_1$ labelling in normal breast (left) and of the substantial up-regulation in breast tumour tissue (right).

10 ## DESCRIPTION OF THE INVENTION

A preferred embodiment of the invention will now be described by way of example only and with reference to the accompanying Figures.

Example 1 - Immunohistochemical Procedure

The immunohistochemical method used in this study was adapted from

15   Barclay [31]. Sections with a thickness of 8 μm were cut from unfixed, frozen tissue using a Reichert Jung 2800 Frigocut cryotome. Sections were air dried at room temperature for 1 hour, fixed for 12 hours in acetone at -20°C and air dried at room temperature for 1 hour prior to antibody labelling. They were then incubated at room temperature with one of either rabbit or sheep anti-$P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$,

20         $P2X_6$ or $P2X_7$ antibody. After washing, sections were then incubated in the secondary antibody; a 1:30 dilution of HRP-conjugated goat anti-rabbit secondary antibody (Dako) for 30 mins for rabbit primaries and HRP-conjugated goat anti-sheep secondary antibody (Dako) for sheep primaries. Slides were again rinsed and then immersed in 15% diaminobenzidine tetrahydrochloride (DAB - Sigma) for 10

minutes. Sections were rinsed, air dried and mounted in DPX (Merck). Control slides were incubated in diluent buffer during the first incubation and then treated in the same manner as the experimental slides. Negative control slides were treated in the same manner as the experimental slides except that the primary antibody was

5 replaced with non-immune serum.

Example 2 - Antibody Production

The consensus sequences of the rat $P2X_1$ [32], $P2X_2$ [33], $P2X_3$ [34], rat $P2X_4$ [35], rat $P2X_5$ [36], rat $P2X_6$ [36], rat $P2X_7$ [37], human $P2X_7$ [38], human $P2X_1$ [39], human $P2X_3$, [40], human $P2X_4$ [41] and human $P2X_5$ [42] cloned receptors were

10 examined for suitable epitopes following the approach adopted in Hansen et al. [15]. The non-homologous epitopes corresponding to the segment Lys199-Cys217 used in rat $P2X_1$ were utilised in rat $P2X_3$, rat $P2X_6$ and rat $P2X_7$. Variations were applied to rat $P2X_4$ which used the sequence Ile235-Gly251 to which was attached a C-terminal Cys residue for cross-linking to a 6 kDa diphtheria toxin domain. The $P2X_2$ epitope

15 was selected from a region within the C1 domain [15], Cys130-Gly153. The rat $P2X_5$ epitope was selected from a region closer to the second transmembrane domain but still extracellular (Lys314-Ile333 to which was added a C-terminal Cys also for conjugation). Although largely homologous with rat $P2X_4$, cross-labelling of $P2X_4$ and $P2X_5$ did not occur. All antibodies against rat sequences were able to label

20 corresponding human receptors. A separate epitope was used for the human $P2X_1$ and $P2X_7$ sequences. This was taken just C-terminal to the first transmembrane domain from Lys68-Val84 with an N-terminal Cys added for conjugation via a diphtheria toxin domain using maleimidocaproyl-N-hydroxysuccinimide. The epitope for

human P2X$_3$ antibody was the equivalent sequence used for rat, while the epitopes for human P2X$_4$ and human P2X$_5$ were Cys270-Asn287 and Cys272-Ser288 respectively. All syntheses were carried out using standard t-BOC chemistry on an ABI synthesiser [43]. The peptide-antigen conjugates were suspended in water at 5

5   mg/mL and aliquots emulsified by mixing with Complete Freund's Adjuvant. Emulsion volumes of 1 mL containing 2 mg of peptide were injected intramuscularly with second, third, fourth and fifth immunisations followed at 2 week intervals using Incomplete Freund's Adjuvant. Final bleeds via venepuncture were obtained at 10-12 weeks, after it was established that adequate antibody titres had been obtained in the

10   rabbits or sheep used for each epitope. The blood was incubated at 37°C for 30 min, and stored at 4°C for 15 h after which the serum was collected following centrifugation and stored at -20°C in small aliquots. Sera were tested with an ELISA assay for antibodies specific for each peptide [15]. The antibody titre, defined as the reciprocal of the serum dilution resulting in an absorbance of 1.0 above background in

15   the ELISA assay, was in the range 75,000±4,000 compared with 225±25 for the pre-immune samples.

Example 3 - Specificity of antibodies

    Each of the P2X antisera used has been shown to possess similar distributions in many cases but with distinctly different distributions in other cases indicating that

20   the antisera do not lack specificity. Specificity was demonstrated by affinity purification of the sera against the cognate peptides. To further verify antibody specificity, individual antibody such as the antibody to P2X$_1$ was added to cells transfected with the corresponding P2X$_1$ cDNA in the presence and absence of a 10mM concentration of the P2X$_1$ epitope. Immunolabelling and confocal imaging of

the tranfected *Xenopus* oocytes demonstrated that the expressed $P2X_1$ is located, as expected, within the cell membrane and the presence of a 10mM concentration of the cognate peptide as an absorption control resulted in the blocking of $P2X_1$ staining [18].

5 **Example 4 - Preparation of tissue for ultrastructural examination of morphology**

Tissue was processed for morphological examination as follows: sections of approximately 3mm X 3mm in size were fixed in 2.5% glutaraldehyde in 0.1M Tris buffer pH 7.2 for 1 hour. They were then washed and post fixed in 2% aqueous osmium tetroxide for 2 hours. After further washing, the tissue was dehydrated in a

10 graded series of alcohols and embedded in Spurr's resin. Curing was carried out at 50^0C for 18 hours. 100nm sections were then cut with a diamond knife, stained with uranyl acetate and Reynolds lead citrate in the usual manner and examined in a Phillips 400 transmission electron microscope.

Example 5 - Ultrastructural Immunocytochemistry

15 The method of Slater [44] was used. In short, thin sections (100nm) were cut and retrieved on 300 mesh nickel grids. After incubation in blocking solution (1%BSA in PBS) for 30 min, the sections were placed on the surface of a drop of the blocking solution (with the addition of 0.05% Tween 20) containing HRP-conjugated goat anti-rabbit secondary antibody or HRP-conjugated goat anti-sheep secondary

20 antibody (diluted 1:100) for 1 h at room temperature. Grids were then rinsed three times for 10 min in PBS and placed on drops of goat anti-rabbit secondary antibody conjugated to 10 nm gold (Nanoprobe) for 1 h at room temperature. The grids were then washed twice with PBS followed by one wash with distilled water, for 10 min each and then placed in the vapour of 2% aqueous osmium tetroxide for 1 minute.

Sections were then stained with aqueous uranyl acetate solution for 20 min, lead

citrate for 10 min, rinsed twice for 10 min in distilled water and examined with a

Phillips 400 electron microscope at 80 kV.

Example 6 – P2X receptors in human cancer tissue

5   In a study of 4 normal and 6 human prostate cancer cases, $P2X_1$, $P2X_3$, and

$P2X_4$ subtypes were markedly increased in human prostate cancer tissue. There was

no labelling at all for these subtypes in normal tissue. The labelling patterns for $P2X_1$

(Figure 1) in the cancerous tissue were particularly interesting in that there was a

greater proportion of labelled acinar epithelial cells with each stage of prostate

10   disease, suggesting a direct correlation between neoplastic transformation and the

extent of $P2X_1$ acinar labelling. $P2X_5$ was also increased in some prostate cancer

cells. There was very little or no labelling for $P2X_5$ in normal tissue.

**Example 7 – P2X receptors, growth, innervation, and metabolic factors, ionic
calcium modulation in young vs aged Wistar rats**

15   <u>P2X receptors and apoptosis:</u>

Studies comparing prostates from four 12 week-old rats and four 1.5 year-old

rats resulted in the detection of a marked increase in epithelial hyperplasia in the aged

rats, resembling BPH in humans (Figure 2). As with the human cancer tissue, $P2X_1$,

$P2X_3$, and $P2X_4$ receptors and tyrosine kinase A receptor antibody were up-regulated

20   in the prostatic epithelium of aged rats, when compared with that of young rats. As

previously discussed, this indicates an increase in protein phosphorylation

(activation), DNA synthesis, intracellular microtubule expression (organelle

transport), up-regulation of adjacent receptors for other neurotransmitters, cell

proliferation and an influx of ions (primarily ionic calcium) into the epithelial cells

indicating apoptosis. An increase in alpha (1B) (voltage-gated calcium channel), and a reduction in the calcium-regulating hormone stanniocalcin was also observed in the aged rat prostates. PDGF and IGF-1 both inhibit apoptosis and were decreased in the aged rats [45]. Thus, the aged rat prostate undergoes apoptosis and similar changes in

5   P2X receptor expression as human prostate cancer tissue, and therefore may be used to investigate prostate cancer aetiology.

Innervation, other receptors and metabolic factors:

In the aged rats, there was an increase in microtubular structures in the fibromuscular septa subjacent to the prostatic epithelium. These structures appeared

10   similar in micrographs depicting the apoptosis-associated purinergic receptors $P2X_1$, $P2X_7$, ionic calcium, and the innervation factors VAMP, muscarinic receptor (M2), SV-2, SNAP-25, S100, and transferrin receptor, all of which were up-regulated in the aged rats. Alpha (1B) voltage-gated calcium channels and tyrosine kinase A receptors were also up-regulated in the aged rats. Stanniocalcin was down-regulated

15   while the $P2X_1$ and $P2X_7$ apoptotic calcium channel receptors were up-regulated. These data indicate an increase of calcium ion inflow, metabolic rate, microtubule transport and innervation of the prostatic epithelium in the aged rats, and also suggest that this model could be used to investigate human prostate cancer.

Example 8 - Breast cancer cell lines

20   In 6 breast cancer cell lines supplied as frozen sections, $P2X_1$, $P2X_3$, and $P2X_4$ purinergic subtypes were labelled using the same techniques employed in the labelling of prostate tissues. The labelling pattern was suggestive of the labelling patterns seen in both human prostate cancer tissue (Figure 2) and the prostate of the male aged Wistar rat (Figure 3).

Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

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DATED this **21st Day of April, 1999**

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1

following figure shows that, compared with prostate epithelium (E) from a young (12 week) rat (' 9), tissue from an aged rat (18 months) shows marked hyperplasia (right)
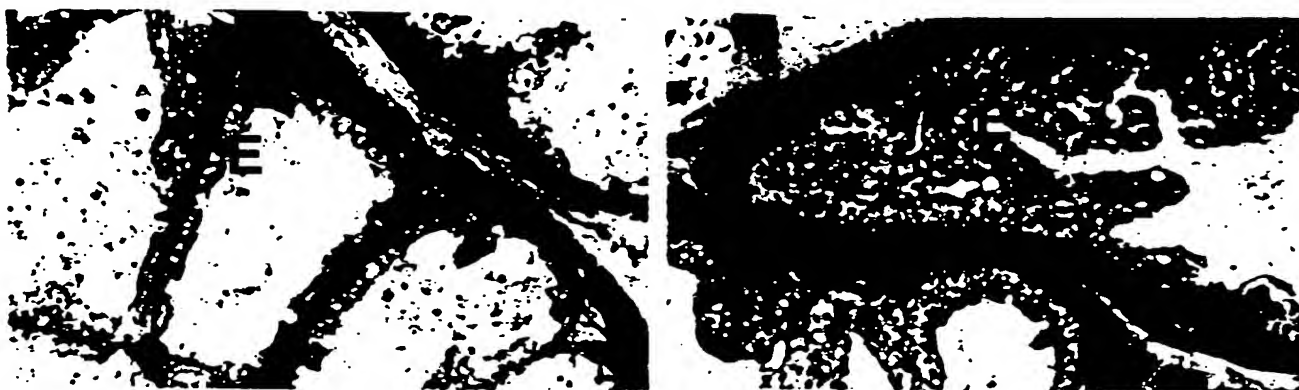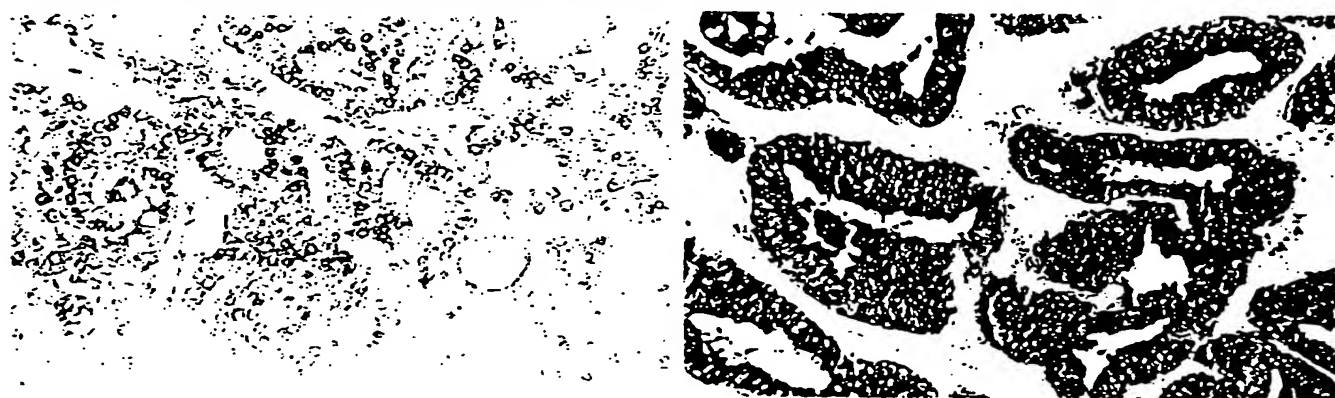

Young Rat   Aged Rat

FIG. 2

The following figure shows an example of the level of $P2X_1$ labeling in a biopsy sample taken from a normal human prostate (left) and from a patient with advanced prostate cancer (right).
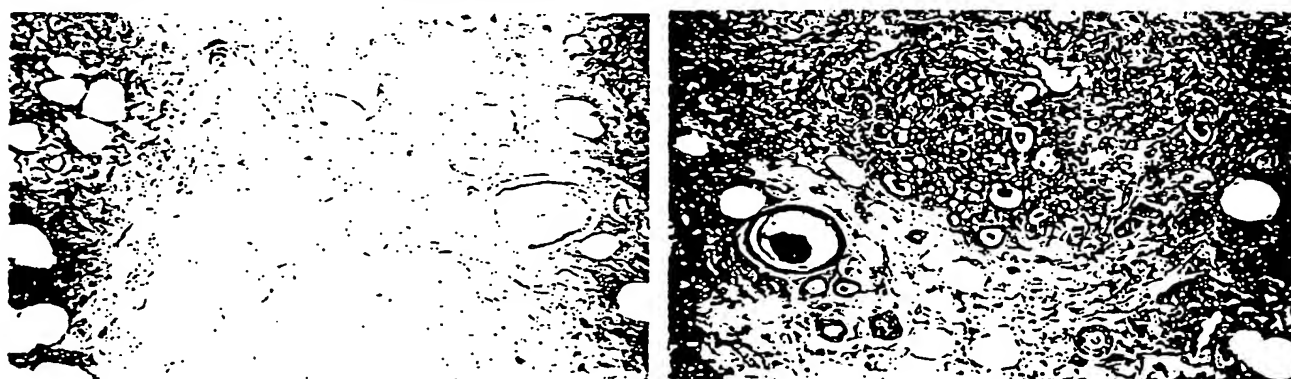

Normal prostate   Prostate Cancer

FIG. 3

The following figure shows an example of $P2X_1$ labeling in normal breast (left) and of the substantial up-regulation in breast tumour tissue (right).

Normal Breast   Breast cancer

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